

1 **INTERPRETIVE SUMMARY**

2 **Relative importance of herd-level risk factors for probability of infection with**  
3 **paratuberculosis in Irish dairy herds**

4 **McAloon**

5 The objective of this study was to identify the most important herd-level risk factors for  
6 Johne's Disease in dairy herds. Analysis of management practices, animal movement and  
7 diagnostic test data from 925 Irish dairy herds identified routine use of the calving area for  
8 sick or lame cows, and length of time spent in the calving pen as the most important risk  
9 factors in Irish dairy herds.

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13 **RISK FACTORS FOR PARATUBERCULOSIS**

14 **Relative importance of herd-level risk factors for probability of infection with**  
15 **paratuberculosis in Irish dairy herds**

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23 **ABSTRACT**

24 Control of paratuberculosis is challenging due to the relatively poor performance of  
25 diagnostic tests, a prolonged incubation period and protracted environmental survival.  
26 Prioritisation of herd-level interventions is not possible because putative risk factors are often  
27 not supported by risk factor studies. The objective for this study was to investigate the  
28 relative importance of risk factors for an increased probability of herd paratuberculosis  
29 infection. Risk assessment data, comprehensive animal purchase history and diagnostic test  
30 data were available for 936 Irish dairy herds. Both logistic regression and a Bayesian beta  
31 regression on the outcome of a Latent Class Analysis were conducted. Population  
32 Attributable Fractions and proportional reduction in variance explained were calculated for  
33 each variable in the logistic and Bayesian models respectively. Routine use of the calving  
34 area for sick or lame cows was found to be a significant explanatory covariate in both  
35 models. Purchasing behaviour for the previous 10 years was not found to be significant. For  
36 the logistic model, length of time calves spend in the calving pen (25%), and routine use of

37 the calving pen for sick or lame animals (14%) had the highest attributable fractions. For the  
38 Bayesian model, the overall R-squared was 16%. Dry cow cleanliness (7%) and routine use  
39 of the calving area for sick or lame cows (6%) and had the highest proportional reduction in  
40 variance explained. These findings provide support for several management practices  
41 commonly recommended as part of paratuberculosis control programmes, however a large  
42 proportion of the observed variation in probability of infection remained unexplained  
43 suggesting other important risks factors may exist.

## 44 INTRODUCTION

45 Bovine paratuberculosis, also called Johne's Disease (**JD**) is characterised by chronic  
46 granulomatous enteritis which manifests clinically as a protein-losing enteropathy causing  
47 diarrhoea, hypoproteinaemia, emaciation and eventually death (Sweeney et al., 2012).  
48 Adverse effects on animal productivity in terms of lower milk yield (McAloon et al., 2016),  
49 higher cull rates (Hendrick et al., 2005), reduced value for culled animals (Richardson and  
50 More, 2009), possible adverse effects on fertility (Johnson-Ifearulundu et al., 2000) and  
51 losses due to continued spread of infection are key drivers in the attempt to control the  
52 disease at farm level. In addition, some research exists to suggest that the aetiologic pathogen  
53 *Mycobacterium avium* subspecies *paratuberculosis* (**MAP**) may pose a zoonotic risk  
54 (Chiodini et al., 2012).

55 Control of JD is difficult due to the relatively poor performance of diagnostic tests (Nielsen  
56 and Toft, 2008), a prolonged incubation period (Sweeney et al., 2011) and protracted  
57 environmental survival (Whittington et al., 2004). Several simulation studies have concluded  
58 that test and cull programmes are unlikely to be effective in isolation and that control of the  
59 disease on farm should centre primarily on closing infection routes, ideally in combination  
60 with testing and culling (Kudahl et al., 2011; Lu et al., 2010; Robins et al., 2015). However,  
61 there is little empirical evidence to support many of the specific interventions introduced at

62 herd level to reduce the probability of introduction and transmission of disease. Although  
63 several risk factor studies have been conducted, results often fail to agree with putative risk  
64 factors that inform key aspects of control programmes, making prioritisation of  
65 implementable control measures difficult (McAloon et al., 2015).

66 At least part of the disparity in these studies may be due to misclassification of positive and  
67 negative herds. Conventionally, herd level risk factor studies are conducted by attributing an  
68 infection status to each herd based on a set number of test reactors. However, such  
69 dichotomised approaches may discard important information regarding the likelihood of  
70 infection and may be biased in larger herds due to imperfect test specificity.

71 The use of Bayesian Latent Class methods allows the estimation of a probability of infection  
72 for each herd conditional on the test characteristics, number of test positive animals and the  
73 total number of animals in the herd (Branscum et al., 2004). In addition, Bayesian methods  
74 account for uncertainty associated with model parameters by modelling each parameter as a  
75 random variable with an associated probability distribution. Bayesian inference allows direct  
76 inference on the parameter of interest, conditional on the observed data and the prior  
77 distributions (Messam et al., 2008).

78 In Ireland, control of non-statutory diseases such as JD is coordinated by Animal Health  
79 Ireland (**AHI**) (More et al., 2011). In 2013, a pilot Voluntary Johne's Disease Control  
80 Programme was introduced which combined annual testing of all animals over 24 months of  
81 age with an on-farm Risk Assessment and Management Plan (**RAMP**) that captured herd  
82 management practices relevant to JD. RAMP has been widely adopted across many countries  
83 with recognisable control programmes (Geraghty et al., 2014). The Risk Assessment (**RA**)  
84 component involves assigning risk scores to different management procedures and areas  
85 based on observations and farmer reported practice. In addition, within the Irish system,

86 animal movement data for the herd is provided for the practitioner to assess bioexclusion  
87 risks. The outcome of the RA is used to inform a Management Plan (**MP**) and, in national  
88 programmes, may have some bearing on herd categorisation or herd risk score.

89 A reduction in animal-level test positivity associated with the implementation of management  
90 practices has been found in a number of small scale investigations on demonstration or study  
91 herds using the RAMP approach (Ferrouillet et al., 2009; Pillars et al., 2011; Espejo et al.,  
92 2012) but progress has not been reproduced in larger studies on commercial farms (Sorge et  
93 al., 2011). Furthermore, to the authors' knowledge there are no studies available investigating  
94 the risk associated with RAMP scores in combination with comprehensive herd purchase  
95 history, or modelling herd level infection status on a probabilistic scale.

96 The objective of this study was to identify and evaluate the relative importance of risk factors  
97 for JD probability of infection using diagnostic test results, RA scores and animal movement  
98 history for herds enrolled in the national voluntary Johne's Disease Control Programme.

## 99 **MATERIALS AND METHODS**

### 100 *Dataset*

101 The dataset for the current study was obtained from herds enrolled in the national voluntary  
102 Johne's Disease control programme. Enrolled herds were required to have all animals that  
103 were 24 months of age and older serologically tested using either serum or milk samples.

104 Diagnostic testing was conducted in approved government and commercial laboratories using  
105 one of 3 commercial ELISA kits approved for use in the AHI programme; Parachek  
106 (Prionics, Switzerland), Paratuberculosis Antibody Screening Test (Idexx, USA) and ID  
107 Screen (IDVet, Montpellier, France). Producers that elect to test using blood or milk sample  
108 were required to test all eligible animals once or twice per year respectively. Test data were  
109 stored centrally in the Irish Cattle Breeding Federation (**ICBF**) computer database. Data were

110 extracted for the period beginning 1st November 2013 and ending 30th December 2014 and  
111 included anonymised cow and herd identifiers, test-date, sample-to-positive (S/P) ratio,  
112 laboratory interpretation (negative, suspect, positive), sample type (blood or milk), testing  
113 laboratory (test kit) and county. Diagnostic test data were available for 1,040 herds.

114 Given that the time frame for extraction exceeded 12 months, several herds had results from  
115 more than one herd screen. To reduce the potential for reverse causality, i.e. the effect of  
116 changes in management occurring as a result of a positive diagnosis, the last herd screen  
117 occurring before the RAMP was preferentially selected, followed by the soonest herd screen  
118 occurring after the RAMP. Test and animal movement data were extracted separately and  
119 datasets were aligned using coded herd identifiers. An additional binary variable was created  
120 to investigate the effect of having the test before (1) versus after (0) the RAMP. The values  
121 for sensitivity (Se) and specificity (Sp) used in the models were appropriate for a single test.

122 In addition to ongoing testing, enrolled herds were required to have an annual RAMP carried  
123 out by a programme approved veterinary practitioner. The RAMP contained questions on the  
124 history of the disease on each farm as well as the risk of introduction of infection from  
125 sources other than animal movement – e.g. colostrum, slurry contractors etc. The  
126 biocontainment component of the RAMP consisted of an additional 28 questions regarding  
127 management practices and observations made on the farm at the day of the visit, which were  
128 deemed to be relevant to the spread of JD.

129 In the RA used in the Irish programme, questions were scored using an ordinal scale of 1, 4, 7  
130 and 10. Within the AHI programme, this method was used to reduce the potential for  
131 subjectivity that might be associated with the use of a continuous scale, since each specific  
132 management practice may be associated with a particular score on the ordinal scale. In  
133 addition, the use of 1, 4, 7 and 10 rather than 1, 2, 3 and 4 was used as a means of weighting

134 the risk associated with each management practice. Higher scores were associated with  
135 increased risk of transmission. However, for this study, RA scores were modelled as  
136 categorical variables, thereby reflecting the risk associated with specific practices rather than  
137 the risk scores *per se* and ensuring that the scale used would have no effect on the model  
138 outcome. Questions asked as part of the RAMP are shown in Table 1.

139 To assist in assessing bioexclusion, the RAMP was pre-populated with animal movement  
140 data for the herd over the preceding 10 years. Movement data included herd size, number of  
141 male and female introductions, number of source herds and number of overseas imports for  
142 every year from 2005 to 2014. Herd sizes less than 20 in 2014 were dropped from the  
143 analysis. Herd size was next summarised across the 10-year period: herds that had a herd size  
144 of <105% of herd size in 2005 were categorised as non-growing herds; the remaining herds  
145 were then broken into mild, moderate and large growth by categorising the percentage growth  
146 into 3 equal quantiles: 5-25%, 26-46% and >46%.

147 Movement and herd size data were then aggregated over two 5-year periods, 2005-2009 and  
148 2010-2014. Within each 5-year period, herds were described as “closed” if no purchases had  
149 been made, herds where no females were purchased and males were purchased at <5% of the  
150 overall herd size were described as “Replacement bulls only”, for the remaining herds, the  
151 number of female purchases was averaged across the 5-year period and broken into 3 equal  
152 quantiles: low, medium and high replacement purchase. Given small number of herds were in  
153 the “closed” category for each 5-year period, this category was combined with “Replacement  
154 bulls only” for the analysis. An additional binary variable was created to identify herds where  
155 males were purchased at greater than 5% of the overall herd size. These herds were  
156 considered likely to be purchasing male animals for beef production in addition to the dairy  
157 enterprise. Finally, the number of source herds purchased from each year was averaged  
158 across each 5-year period.

159 Herds were removed from the dataset when one or more of the 3 components of the scheme:  
160 diagnostic test results, herd movement history and RAMP results, were missing or  
161 incomplete. The final dataset included data from 925 herds.

## 162 *Analytical Models*

163 ***Model 1; Logistic regression analysis.*** The outcome variable was herd infection  
164 status (positive or negative) and herds were defined as positive when they had 2 or more  
165 cows with positive tests. A cut point of 2 positive cows was used to account for imperfect test  
166 specificity; with herd sizes represented in this study, it was less likely that two positive results  
167 would both be false positives. In addition, for the purposes of comparison, the final model  
168 was reassessed with a cut-point of 1 reactor, the final single reactor model is included as  
169 supplementary material (Supplementary Material 1 - Table 1). Data analysis was conducted  
170 in R-studio version 1.0.44 (The R Core Team, 2016). Individual explanatory covariates were  
171 initially investigated within a univariable logistic regression framework and carried forward  
172 for multivariable regression analysis when  $P < 0.2$ . Before addition to the multivariable model,  
173 covariates were assessed for correlation. When 2 variables were highly correlated ( $> 0.8$ ), one  
174 was selected and brought forward for multivariable analysis based on whichever variable  
175 resulted in the model with the lowest Akaike Information Criterion (**AIC**). Variables dropped  
176 due to collinearity were replaced into the final model to test for significance. The model was  
177 constructed using a forward stepwise elimination and variables with a significance  
178 probability  $P < 0.05$  were retained in the model. Herd size and test medium were forced into  
179 the model from the beginning of the multivariate analysis to account for the potential  
180 confounding effect of these variables on test sensitivity and specificity. In addition, for the  
181 purpose of comparison, the model was reconstructed using the AIC solely as the selection  
182 criteria. Finally, the Population Attributable Fraction (**PAF**) was calculated for each variable  
183 in the model based on distribution of exposure in cases (Hanley, 2001);



$$184 \quad \text{PAF} = \frac{\text{RR}-1}{\text{RR}} \times \frac{\text{number of exposed cases}}{\text{overall number of cases}} \quad (1)$$

185 Adjusted relative risks were calculated from the Odds Ratios of the final model using the  
 186 method described by Zhang (1998).

187 **Model 2; Bayesian analysis.** This analysis was conducted in two stages. First, a  
 188 probability of infection for each herd was estimated using a Bayesian latent class model. This  
 189 model had the same structure, and was implemented using the same methods as described in  
 190 McAloon et al.(2016a). Briefly, the number of test positive animals in a given herd was  
 191 assumed to follow a binomial distribution with a probability equal to the apparent prevalence  
 192 and n equal to the number of animals tested. The apparent prevalence (**AP**) was related to the  
 193 true prevalence (**TP**) and the test sensitivity (**Se**), and specificity (**Sp**) by the formula;

$$194 \quad \text{AP} = \text{TP} \times \text{Se} + (1 - \text{TP}) \times (1 - \text{Sp}) \quad (2)$$

195 TP was modelled as a mixture of a Bernoulli distribution, with a probability equal to the  
 196 probability of infection for the herd, and a beta distribution equal to the within herd true  
 197 prevalence.

198 In the second step, the mean probability of infection for each herd was used as the outcome  
 199 variable in a Bayesian beta regression model with a logit link (Branscum et al., 2007). The  
 200 model was built using a forward stepwise analysis and variables were retained in the model  
 201 when the 95% credible interval did not include zero.

202 The model had the following structure;

$$203 \quad \mu_i \sim \text{beta}(a_i, b_i) \quad (3)$$

$$204 \quad a_i = \psi_i \times \gamma \quad (4)$$

$$205 \quad b_i = (1 - \psi_i) \times \gamma \quad (5)$$

206  $\text{logit}(\psi) \leftarrow \beta_0 + \beta_1 X_1 \dots$  (6)

207  $\gamma \sim \text{gamma}(G_1, G_2)$  (7)

208 where  $\mu_i$  was the probability of infection for the  $i$ -th herd which was modelled by a beta  
 209 distribution. To facilitate incorporation of the covariate information into the regression  
 210 model, the beta distribution was parameterised in terms of its mean,  $\psi$ , and a parameter  
 211 related to its variance,  $\gamma$  (Bransum et al., 2007). A logit link was used to estimate the  
 212 regression coefficient,  $\beta$ , for each covariate,  $X$ .  $G_1$  and  $G_2$  were the shape and scale  
 213 parameters for the gamma distribution,  $\gamma$ . Larger values of  $\gamma$  correspond to less heterogeneity  
 214 in the data. For this analysis, a prior gamma distribution with a low mean and high variance  
 215 was used ( $G_1=G_2=0.01$ ).

216 Diffuse normal distributions (mean = 0, precision = 0.01) were used for the priors of each  
 217 coefficient in the model. Model outcomes for each covariate were reported as probability of  
 218 infection by converting the coefficients according to the formula;

219 
$$p = \frac{1}{1 + e^{-(\beta_0 + \sum \beta_j X_j)}}$$
 (8)

220 Where  $p$  is the probability of infection,  $\beta_0$  is the intercept and  $\beta_j$  is the coefficient of the  $j$ -th  
 221 covariate,  $X_j$  (Dohoo et al., 2010). To fit the model, mean probabilities of infection less than  
 222 0.01 ( $n=8$ ) were rounded to 0.01 and those greater than 0.99 ( $n=100$ ) to 0.99.

223 Model fit was assessed using posterior predictive simulations (Gelman et al., 2000). The  
 224 predictive simulation incorporated within the model was;

225  $\text{Pred}\mu_i \sim \text{beta}(a_i, b_i)$  (9)

226  $\text{Pred}\mu_i$  was monitored for the final 5000 iterations of the overall simulation. Predicted  
 227 probability of infection was compared to the probability of infection outcome from the latent  
 228 class model and the mean difference and the mean squared difference was used to compare

229 models. The proportional reduction in variance explained was calculated for each variable by  
230 removing each variable in turn from the full model, re-estimating model parameters and,  
231 calculating the difference in R-squared relative to the full model.

232 The model was implemented in WinBUGS 1.4.1 (Lunn et al., 2000), the first 5,000 iterations  
233 were discarded as burn in, by which time convergence had occurred, and 15,000 iterations  
234 used for posterior inference. Convergence was assessed by visual assessment of the chain as  
235 well as by running multiple chains from dispersed starting values (Christensen et al., 2012).  
236 The code for analysis is provided as supplementary material (Supplementary Material 2).

237

238

## RESULTS

### 239 *Descriptive Statistics*

240 925 herds were present in the final dataset. Overall median herd size was 80, Using a cut-  
241 point of 2 reactors, 265 herds were positive, giving an apparent prevalence of 0.29. RAMP  
242 scores are summarised in Figure 1 and animal purchase data for the 10 years prior to  
243 diagnostic testing are summarised in Table 2.

244 Median herd growth from 2005 to 2015 was 25%. From 2005-2009 only 30 herds were  
245 classified as closed, with a further 70 herds classified as replacement bull purchases only.  
246 Similarly, from 2010-2014, 37 and 109 herds were closed or replacement bull only. From  
247 2005-2009, 30% of herds purchased replacement females at an annual average of more than  
248 7.5% of the total herds size, whilst from 2010-2014, the equivalent figure was 28%. In each  
249 5-year block the mean annual number of source herds was more than 1 for 54% of the herds  
250 in 2005-2009 and 38% in 2010-2014.

251 **Model Outcomes**

252 **Model 1; Logistic regression.**

253 The outputs from the final multivariable logistic regression model are shown in Table 3. The  
254 reference category for each variable has been selected to avoid negative coefficients. Herd  
255 size was positively associated with herd positivity with an odds ratio of 1.01 for each  
256 additional animal. Herds testing with milk were 1.57 times as likely to test positive as those  
257 testing with blood. A large seasonal effect was apparent with herds testing in January 2.1  
258 times as likely to test positive as those tested in May. Herds where pooled colostrum was  
259 used for more than 10% of the calves were 2.1 times as likely to be positive compared to  
260 those herds where calves were fed colostrum from their own test-negative dam, this category  
261 had a PAF of 11.6%. Herds where weaned heifers were grazed near adult animals, but  
262 without direct or indirect contact were 1.7 times as likely to test positive, and had a PAF of  
263 10.5%, as those where direct or indirect contact was possible. Herds where the milking cow  
264 environment had clearly visible manure contamination were 1.7 times as likely to be defined  
265 as positive compared to herds where only trace amounts of manure were visible with a PAF  
266 of 7.6%. Herds where the calving area was routinely used for housing sick and lame cows  
267 were 2.2 times as likely to be positive than those where the calving area was never used for  
268 non-calving cows and had a PAF of 14.2%. Herds where more than 50% of the calves were  
269 removed from the dam within 30 minutes of birth were 2.3 times as likely to test positive as  
270 those where 90% of the calves were removed within 15 minutes of birth, the PAF of this  
271 variable was 24.7%. Finally, herds that experienced small growth (5-25%) were 1.7 times as  
272 likely to test positive as those that expanded to a high (>50%) growth in herd size.

273 **Model 2; Bayesian Model.** Outputs from the final Bayesian beta-regression model are  
274 shown in Table 4. Overall the model explained 16% of the variation, indicating that a  
275 considerable amount of the variation in the probability of a herd being positive remained

276 unexplained. The reported presence of previous clinical or test positive animals was  
277 responsible for 22.6% of the overall variance explained (R-squared) and resulted in a mean  
278 probability of infection (95% probability interval) of 0.72 (0.66-0.77). A strong seasonal  
279 effect was again observed which was responsible for 35% of the overall R-squared, with  
280 herds testing in January having a probability of infection of 0.77 (0.69, 0.83). The proportion  
281 of the herd tested comprised 3.2% of the overall R-squared and was negatively associated  
282 with the probability of infection. The probability of infection dropped by 5% with each  
283 additional 10% of the herd tested. Feeding of forages to weaned heifers that had been spread  
284 with slurry in the previous season increased the probability of infection by 8% (0-16%). Dry  
285 cow cleanliness comprised 7.1% of the overall R-squared and herds where dry cows had no  
286 faecal contamination visible had a mean probability of infection of 0.67, compared to 0.60 in  
287 herds where faecal contamination was visible on the legs but not extending above the  
288 dewclaws. The use of the calving pen for non-calving animals comprised 5.8% of the overall  
289 R-squared and herds where the calving pen was routinely used for sick or lame animals had a  
290 probability of infection of 0.69 (0.65-0.74).

## 291 **DISCUSSION**

292 The present study used a combination of frequentist and Bayesian methods to investigate risk  
293 factors for positivity and infection probability in Irish dairy herds using data collected as part  
294 of the AHI voluntary programme.

295 In the logistic regression model, the speed with which calves were removed from the calving  
296 pen was the most important variable (PAF = 24.7%). Herds in which >90% calves were  
297 removed within 15 minutes of birth had the lowest risk of being positive, with herds where  
298 calves were still removed within 30 minutes were 2.2 times as likely to be positive. In this  
299 case the large PAF is caused by a combination of the relatively large odds ratio, combined

300 with the large proportion of herds within the higher risk category (n=507). The practice of  
301 removing the calf immediately from the dam is commonly advocated for the purpose of  
302 paratuberculosis control however, despite investigating this risk factor, a number of studies  
303 have failed to find this practice associated with an increased risk of positivity (Johnson-  
304 Ifearulundu and Kaneene, 1998; Wells and Wagner, 2000; Nielsen and Toft. 2011).  
305 However, Cashman et al. (2008), found an increased risk of culturing MAP from milk filters  
306 in herds where a greater proportion of calvings were not supervised. Interestingly, the  
307 practice of immediate separation from the dam is also recommended for the control of calf  
308 diseases (McGuirk and Collins, 2004), although studies into the benefit of calf removal have  
309 been equivocal (Weary and Chua, 2000; Trotz-Williams et al., 2007), McAloon et al., 2016b  
310 recently found improved passive transfer in calves removed immediately from the calving  
311 pen, compared with those spending more than 30minutes with the dam.

312 The use of the calving pen to house sick or lame animals was the second most important  
313 management factor in both the Bayesian and logistic model with a proportional reduction in  
314 R-squared of 5.8% and a PAF of 14.2%). Herds that routinely used the calving pen for sick or  
315 lame cows had a mean probability of infection of 0.69 (0.65-0.74) and were 2.3 times as  
316 likely to be defined as positive compared with herds in which the calving pen was never used  
317 for sick or lame cows. The use of the calving pen for sick or lame cows is often discouraged  
318 as part of JD control programmes (Sweeney et al., 2012). This is based on the rationale that  
319 cows that are subclinically infected with JD are more likely to be susceptible and therefore  
320 affected with other diseases but there appears to be little empirical evidence to support this  
321 claim. It is however likely that “sick” cows would also include those suffering from  
322 symptoms of clinical JD and this practice could facilitate disease transmission. In addition,  
323 routine use of the calving pen for sick animals could be an indicator of increased stocking

324 density and insufficient building space, potentially resulting in increased exposure of calves  
325 to infected faecal material.

326 The source of colostrum was significant in the logistic regression model and had a PAF  
327 11.6%). However, this variable was not significant in the Bayesian model. Herds in which  
328 over 10% of calves were fed colostrum from sources other than the dam (Risk Score 10) were  
329 2.1 times as likely to be defined positive compared with herds in which dam-only colostrum  
330 was practiced. Nielsen et al. (2008) found that calves fed colostrum from multiple sources  
331 were 1.2 times as likely to be positive than those fed dam-only colostrum. However, this  
332 finding is not consistent. For example, in a longitudinal study, Pithua et al. (2011) found that  
333 calves fed PCR-positive colostrum were not at a significantly greater risk of testing positive  
334 as adults compared to those fed PCR-negative colostrum. In contrast, the same author found  
335 that calves fed a commercial colostrum replacer were less likely to be identified as positive as  
336 adults than those fed conventional colostrum (Pithua et al., 2009). Similarly, Stabel (2008)  
337 found that colostrum pasteurisation reduced the incidence of disease in calves as measured by  
338 interferon gamma. However, in the long-term, risk of infection for this cohort of calves was  
339 not different between groups (Godden et al., 2015).

340 Dry cow cleanliness was significantly associated with probability of infection in the Bayesian  
341 model and was responsible for a reduction in R-squared of 7.1%). The finding that the lowest  
342 dry cow contamination score was associated with an increased risk of infection compared to  
343 the second lowest score seems counterintuitive. This finding could potentially be explained  
344 given the seasonal calving system operated on Irish dairy herds, i.e. the fact that the dry cow  
345 pen is not in use for a large majority of the year. However, whenever the month when the  
346 RAMP was conducted was forced into the model, the variable remained significant  
347 suggesting that the time when the RAMP was conducted was not confounding this variable.

348 It is worth noting that risk scores of 7 and 10 were associated with increased risk compared to  
349 risk score 4 although these associations were not significant.

350 Similarly, the finding that herds where heifers were housed or grazed near cows but had no  
351 direct contact (Q23) were at greater risk of testing positive compared with those where there  
352 was direct contact or heifers were exposed via run-off or slurry spreading, is difficult to  
353 explain. The susceptibility to infection has been shown to decrease with age (Windsor and  
354 Whittington, 2010), however, more recently, Mortier et al. (2013) demonstrated that calves  
355 up to the age of 12 months could be infected with both high and low doses of MAP. Despite  
356 been identified as the lowest risk category for this model, the large proportion of herds where  
357 weaned heifers had direct or indirect contact with adult cows (45%) is a significant concern.

358 The milking cow environment score had a PAF of 7.6% with herds where manure was clearly  
359 visible were 1.7 times as likely to test positive as those where trace amounts of manure was  
360 visible. Although infection of adult animals is possible with sufficiently high doses of MAP  
361 (Whittington and Windsor, 2010), in this case it is more likely that the finding is indicative of  
362 the overall hygiene of the farm, rather than the specific risk to adult animals *per se*.

363 In the Bayesian model, the feeding of forages that had received slurry from adult animals was  
364 significantly associated with the probability of infection, however this variable only  
365 comprised 1.3% of the overall variation. Interestingly, a similar finding was observed in a  
366 North American study (Obasanjo et al., 1997). In contrast, Kohl et al. (2010) was unable to  
367 culture MAP from baled grass silage following inoculation, although samples were positive  
368 by PCR. The authors in that study suggested that conserved forages constituted a minor risk  
369 for transmission. In a pasture based system where conserved forages are consumed during the  
370 housed period, avoiding the use of slurry on harvested grass may difficult to avoid, which is  
371 reflected in the high proportion of herds in the higher risk category (95%). In addition, on



372 many farms, avoiding spreading slurry on grass harvested for younger animals would  
373 necessitate segregation of conserved forage for different age groups of animals. Furthermore,  
374 increased application on adult ground would lead to an increase in potassium content (Soder  
375 and Stout, 2003), resulting in an increased Dietary Cation Anion Difference and therefore an  
376 increased risk of hypocalcaemia (Goff, 2004).

377 The change in herd size from 2005 to 2014 was only significant in the logistic model with a  
378 PAF of 6.7%. In that case, the lowest risk of testing positive was observed in herds that had  
379 undergone significant expansion (>50%) over the 10-year period. Anecdotally, herd  
380 expansion has been associated with an increased risk of poor health in general. However, in a  
381 previous Irish study, Jago and Berry (2011) found improved reproductive performance in  
382 dairy herds undergoing higher levels of expansion suggesting that this finding could be  
383 confounded by improved management in general on these farms. In addition, the same study  
384 found that the average parity number decreased in herds as the rate of expansion increased.  
385 The sensitivity of the ELISA is known to increase with increased age (Nielsen et al., 2013),  
386 therefore as the mean age of the herd decreases, the effective herd level sensitivity of the  
387 ELISA screen is also likely to have decreased.

388 In the Bayesian model, previous presence of test positive or clinical cases of JD explained the  
389 largest proportion of variance explained (41%), however, in the logistic model, this variable  
390 had a PAF of 12.6%. The finding is unsurprising and highlights awareness of the herd  
391 infection status in many herds. It was decided to couple this variable with whether or not the  
392 RAMP had been conducted prior to or after the herd screen in an attempt to remove any  
393 possible confounding associated with prior knowledge of the disease in the herd. When the  
394 variable was removed from the model, all of the variables remained significant.

395 Given the imperfect specificity of the test, herd size was included as a variable, largely to  
396 account for confounding since larger herds would have an inherently greater risk of having  
397 false positive test results. In agreement with this, herd size was found to be significant in the  
398 logistic model, whereas in the Bayesian model, herd size was not significant. However,  
399 previous studies have documented increased risk of infection status in association with  
400 increased herd size. Collins (1994) found that larger herds in Wisconsin were more likely to  
401 be defined positive based on serological methods, however this association was not  
402 statistically significant. Similarly, Daniels et al., (2002) found that clinical disease was more  
403 likely to be present on Scottish farms in the preceding 10 years when herd size was larger.  
404 Finally, based on analysis of submitted laboratory samples, Barrett et al. (2011), found a  
405 significant association between herd positivity and herd size.

406 To the authors' knowledge this study represents the first use of herd level outputs from a  
407 Bayesian latent class model to fit a beta regression on herd level risk factors. Furthermore,  
408 the use of PAF from a classical logistic regression model has not yet been used to investigate  
409 the relative importance of risk factors for paratuberculosis. The Bayesian model reduced the  
410 risk for misclassification due to imperfect test performance as test Se and Sp were  
411 incorporated within the latent class model. On the other hand, the logistic model was based  
412 on the binary outcome of assigned herd status facilitated the use of PAF, giving a more  
413 intuitive impression of the relative importance of significant risk factors. Both methods are  
414 limited by the sampling method in this study. The Irish JD control programme is voluntary  
415 and therefore may not be representative of the average Irish dairy farm. In addition, the study  
416 utilised a cross sectional design based on a single test, single RA strategy. Although the  
417 recommendation from the national programme is to conduct the RA prior to testing, it is  
418 possible that testing may have been conducted prior to the completion of the RA, prompting  
419 the introduction of management changes and thereby introducing the risk of reverse causality

420 into the analysis. The authors attempted to reduce this risk by using RA data from the first  
421 year of the programme. Given that the management practices identified as significant in this  
422 are biologically plausible and largely agree with putative risk factors, it seems unlikely that  
423 reverse causality was a significant issue in this analysis.

424 An unforeseen, outcome of the analysis was the strong seasonal effect that was observed in  
425 both models. In each model, January, February and March were associated with a greater risk  
426 of positivity. The risk decreased in April, May and June before increasing again in the  
427 autumn and winter. Within the Irish system, seasonality has the potential to be confounded by  
428 stage of lactation and therefore milk yield. Nielsen and Toft (2012), found that the risk of  
429 being test positive on milk ELISA was greatly increased in the first 7 DIM and increased  
430 linearly over the course of the lactation after correcting for milk yield which appeared to have  
431 a diluting effect. To investigate the current dataset further, we separated the dataset into herds  
432 using milk and those using blood. Although the lowest risk month for both datasets was the  
433 same, i.e. May, different temporal trends were apparent depending on the test media used. In  
434 the milk dataset, the risk steadily increased from March to August with a large peak in  
435 September before declining again from September to December. With the serum dataset, the  
436 highest risk of test positivity was in January with a decline until May, with a second smaller  
437 peak in July. These findings require further investigation to examine whether this trend  
438 repeats in subsequent years.

## 439 **CONCLUSIONS**

440 This study demonstrates the use of PAF and Bayesian beta-regression as a means of  
441 investigating the relative importance of herd-level interventions on a national scale for the  
442 control of paratuberculosis. The findings of this study suggest that the national control  
443 programme should emphasise avoiding the use of the calving pen to house sick and/or lame

444 cows, reducing the length of time calves spend in the calving pen to less than 15 minutes and  
445 reducing the prevalence of pooled colostrum feeding as key interventions to reduce the  
446 prevalence of paratuberculosis in Irish dairy herds. It should also be noted however, that a  
447 large proportion of the observed variation in probability of infection remained unexplained  
448 suggesting other important risks factors may exist.

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561 **Table 1.** Questions asked as part of on-farm Risk Assessment (**RA**) conducted on 925 dairy  
562 herds enrolled in the Irish national Johne's Disease Control Programme

|                                                                                                                                     |
|-------------------------------------------------------------------------------------------------------------------------------------|
| Q1. Have you ever completed a Johne's Disease herd test?                                                                            |
| Q2. Has there been any suspect cases of JD on the farm?                                                                             |
| Q3. Have you had any confirmed clinical JD or test positive cows in your herd?                                                      |
| Q4. Do you use your own equipment to spread slurry on your farm?                                                                    |
| Q5. Do you spread cattle/slurry from other herds on your pasture?                                                                   |
| Q6. Do you graze cattle purchased by you for fattening on your pasture?                                                             |
| Q7. Do you graze cattle/cows on commonage or with cattle from other herds                                                           |
| Q8. Do you graze on rented ground?                                                                                                  |
| Q9. Do you use contract rearers or rear calves/heifers under a different herd number?                                               |
| Q10. Do sheep cograze on this farm?                                                                                                 |
| Q11. Are calves fed colostrum from own mother or from known low risk colostrum cows or artificial                                   |
| Q12. Are at least 3 litres of colostrum (first milking) consumed within the first 2 hours?                                          |
| Q13. Are calves fed on low risk whole milk                                                                                          |
| Q14. How often is non-saleable whole milk (high risk) fed?                                                                          |
| Q15. Are calves housed in individual or group pens in the first week?                                                               |
| Q16. Is there exposure to cow manure in the calf housing or grazing area?                                                           |
| Q17. Is there exposure to cow manure by watering or feeding utensils?                                                               |
| Q18. Are calves fed forages that have received slurry from adult animals within the last year?                                      |
| Q19. Do you feed or have you fed colostrum from other herds?                                                                        |
| Q20. When was this last fed?                                                                                                        |
| Q21. Do you feed milk from cows from other herds                                                                                    |
| Q22. When was this last fed?                                                                                                        |
| Q23. Are weaned heifers exposed to cows or their manure at any time?                                                                |
| Q24. Are maiden heifers exposed to cows or their manure at any time?                                                                |
| Q25. What is the overall hygiene and cleanliness score of weaned heifers                                                            |
| Q26. What is the overall hygiene and cleanliness score of maiden or incalf heifers?                                                 |
| Q27. Are weaned heifers ( $\geq 6$ months) fed forages that have received slurry from adult animals within the last year?           |
| Q28. Are maiden or incalf heifers ( $\geq 6$ months) fed forages that have received slurry from adult animals within the last year? |
| Q29. Dry cow area environment hygiene score                                                                                         |
| Q30. Milking cow area environment hygiene score                                                                                     |
| Q31. Dry cow cleanliness                                                                                                            |
| Q32. Milking cow cleanliness                                                                                                        |
| Q33. Single or multiple cows in calving areas?                                                                                      |
| Q34. Manure build up                                                                                                                |
| Q35. Manure on soiled udders and legs of cows?                                                                                      |
| Q36. Calving area used for lame or sick cows?                                                                                       |
| Q37. Calving area used for JD clinical or JD test positive cows?                                                                    |
| Q38. Birth of calves in areas other than designated calving area?                                                                   |

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Q39. Likelihood of calf nursing cow(s)?

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Q40. How fast are newborn dairy calves removed from their mothers?

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566 **Table 3.** Summary of herd-level characteristics and animal introduction data for 925 dairy  
 567 herds enrolled in the Irish national Johne's Disease Control Programme. Definition of  
 568 categories and proportion of herds defined as positive based on  $\geq 2$  animals testing positive.

| <b>Variable</b>                                        | <b>Number in category</b> | <b>Percent in category</b> | <b>Number positive</b> | <b>Percent positive</b> |
|--------------------------------------------------------|---------------------------|----------------------------|------------------------|-------------------------|
| <b>Herd size</b>                                       |                           |                            |                        |                         |
| $\leq 60$                                              | 246                       | 27%                        | 70                     | 17%                     |
| 61-80                                                  | 217                       | 23%                        | 66                     | 23%                     |
| 81-116                                                 | 232                       | 25%                        | 61                     | 31%                     |
| $>116$                                                 | 230                       | 25%                        | 101                    | 44%                     |
| <b>Test medium</b>                                     |                           |                            |                        |                         |
| Blood                                                  | 588                       | 64%                        | 166                    | 28%                     |
| Milk                                                   | 337                       | 36%                        | 99                     | 29%                     |
| <b>Test precedes RAMP<sup>1</sup></b>                  |                           |                            |                        |                         |
| Yes                                                    | 493                       | 53%                        | 157                    | 32%                     |
| No                                                     | 432                       | 47%                        | 108                    | 25%                     |
| <b>Herd growth 2005-2014</b>                           |                           |                            |                        |                         |
| $<5\%$                                                 | 232                       | 25%                        | 56                     | 24%                     |
| 5-25%                                                  | 219                       | 24%                        | 66                     | 30%                     |
| 26-46%                                                 | 218                       | 24%                        | 67                     | 31%                     |
| $>45\%$                                                | 256                       | 28%                        | 76                     | 30%                     |
| <b>Mean annual purchases 2005 – 2009<sup>2</sup></b>   |                           |                            |                        |                         |
| Closed/Replacement Bulls Only                          | 101                       | 11%                        | 28                     | 28%                     |
| Females at $<2\%$ of herd size                         | 266                       | 29%                        | 81                     | 30%                     |
| Females at 2-7.5% of herd size                         | 281                       | 30%                        | 72                     | 26%                     |
| Females at $>7.5\%$ of herd size                       | 277                       | 30%                        | 84                     | 30%                     |
| <b>Mean number of herds purchased from 2005 - 2009</b> |                           |                            |                        |                         |
| $<0.4$                                                 | 240                       | 26%                        | 70                     | 29%                     |
| 0.4-1.0                                                | 180                       | 19%                        | 51                     | 28%                     |
| 1.0-2.2                                                | 234                       | 25%                        | 71                     | 30%                     |
| $>2.2$                                                 | 271                       | 29%                        | 73                     | 27%                     |
| <b>Beef purchases 2005-2009<sup>3</sup></b>            |                           |                            |                        |                         |
| Yes                                                    | 383                       | 41%                        | 107                    | 28%                     |

|                                                      |     |     |     |     |
|------------------------------------------------------|-----|-----|-----|-----|
| No                                                   | 542 | 59% | 158 | 29% |
| <b>Mean number of herds purchased from 2010-2014</b> |     |     |     |     |
| Closed/Replacement Bulls Only                        | 146 | 16% | 43  | 29% |
| Females at <2% of herd size                          | 316 | 34% | 87  | 28% |
| Females at 2-7.5% of herd size                       | 204 | 22% | 57  | 28% |
| Females at >7.5% of herd size                        | 259 | 28% | 78  | 30% |
| <b>Beef purchases 2010-2014</b>                      |     |     |     |     |
| Yes                                                  | 327 | 35% | 95  | 29% |
| No                                                   | 598 | 65% | 170 | 28% |
| <b>Mean number of herds purchased from 2010-2014</b> |     |     |     |     |
| <0.4                                                 | 363 | 39% | 98  | 27% |
| 0.4-1.0                                              | 209 | 23% | 56  | 27% |
| 1.0-2.2                                              | 195 | 21% | 63  | 32% |
| >2.2                                                 | 158 | 17% | 48  | 30% |

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570 <sup>1</sup>RAMP = Risk Assessment and Management Plan

571 <sup>2</sup>Replacement Bulls Only = herds not introducing females and only introducing males at  $\leq 5\%$   
572 of the overall herd size each year

573 <sup>3</sup>Beef purchases = herds purchasing males at  $>5\%$  of the overall herd size each year

574 **Table 4.** Results from multivariable logistic regression model assessing the association  
 575 between RA questions, animal movement data and the outcome “herd positivity”, defined as  
 576 herds with 2 or more positive animals in the herd

| Variable                                                                                       | n <sup>1</sup> | Coefficient | Odds Ratio | 95% Confidence Intervals | p      | PAF <sup>2</sup> |
|------------------------------------------------------------------------------------------------|----------------|-------------|------------|--------------------------|--------|------------------|
| Herd Size                                                                                      |                | 0.01        | 1.01       | 1.01, 1.01               | <0.001 |                  |
| <b>Test Medium</b>                                                                             |                |             |            |                          |        |                  |
| Milk                                                                                           | 337            | 0.45        | 1.57       | 1.57, 1.57               | 0.021  | 9.7%             |
| Blood                                                                                          | 588            | REF         |            |                          |        |                  |
| <b>Test Month</b>                                                                              |                |             |            |                          |        |                  |
| January                                                                                        | 48             | 2.12        | 8.33       | 3.61, 19.24              | <0.001 | 5.5%             |
| February                                                                                       | 53             | 1.69        | 5.42       | 2.34, 12.57              | <0.001 | 4.9%             |
| March                                                                                          | 69             | 1.34        | 3.82       | 1.77, 8.23               | 0.001  | 4.4%             |
| April                                                                                          | 114            | 0.66        | 1.93       | 0.96, 3.91               | 0.064  | 3.2%             |
| May                                                                                            | 160            | REF         |            |                          |        |                  |
| June                                                                                           | 129            | 0.38        | 1.46       | 0.73, 2.92               | 0.277  | 2.0%             |
| July                                                                                           | 91             | 1.53        | 4.62       | 2.35, 9.08               | <0.001 | 8.2%             |
| August                                                                                         | 67             | 1.23        | 3.42       | 1.59, 7.38               | 0.002  | 4.0%             |
| September                                                                                      | 53             | 1.03        | 2.8        | 1.21, 6.5                | 0.016  | 2.6%             |
| October                                                                                        | 69             | 1.52        | 4.57       | 2.17, 9.64               | <0.001 | 5.5%             |
| November                                                                                       | 44             | 1.69        | 5.42       | 2.33, 12.61              | <0.001 | 4.2%             |
| December                                                                                       | 28             | 1.19        | 3.29       | 1.16, 9.31               | 0.026  | 1.5%             |
| <b>Q3. Presence of clinical JD or test positive cows in past<sup>3</sup></b>                   |                |             |            |                          |        |                  |
| No and RA conducted after testing                                                              | 257            | REF         |            |                          |        |                  |
| No and RA conducted before testing                                                             | 348            | 0.38        | 1.46       | 0.91, 2.35               | 0.114  | 7.8%             |
| Yes and RA conducted after testing                                                             | 175            | 1.03        | 2.8        | 1.67, 4.69               | <0.001 | 12.2%            |
| Yes and RA conducted before testing                                                            | 145            | 1.21        | 3.35       | 1.92, 5.84               | <0.001 | 12.6%            |
| <b>Q11. Are calves fed colostrum from own mother or from known low risk colostrum sources?</b> |                |             |            |                          |        |                  |
| Calves receive colostrum from their own test negative mother                                   | 291            | REF         |            |                          |        |                  |
| Calves receive colostrum from their own mother (no selection)                                  | 278            | 0.39        | 1.48       | 0.94, 2.31               | 0.088  | 6.9%             |
| 1-10% of calves receive colostrum from source other than dam                                   | 166            | 0.35        | 1.42       | 0.85, 2.38               | 0.190  | 4.2%             |

|                                                             |     |      |     |            |       |       |
|-------------------------------------------------------------|-----|------|-----|------------|-------|-------|
| >10% of calves receive colostrum from source other than dam | 190 | 0.74 | 2.1 | 1.28, 3.42 | 0.003 | 11.6% |
|-------------------------------------------------------------|-----|------|-----|------------|-------|-------|

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**Q23. Are weaned heifers exposed to cows or their manure at any time?**

|                                                                                                                                                      |     |      |      |            |       |       |
|------------------------------------------------------------------------------------------------------------------------------------------------------|-----|------|------|------------|-------|-------|
| Never housed/grazed with adult animals, no direct contact and no exposure to manure. Not fed uneaten rations from cows and not sharing water troughs | 241 | 0.27 | 1.31 | 0.85, 2.02 | 0.212 | 4.1%  |
| Housed/grazed near cows but no direct or indirect contact                                                                                            | 269 | 0.54 | 1.72 | 1.15, 2.55 | 0.007 | 10.5% |
| Housed/grazed near cows, direct or indirect contact possible                                                                                         | 415 | REF  |      |            |       |       |

---

**Q30. Milking cow environment hygiene score**

|                                                                              |     |      |      |            |       |      |
|------------------------------------------------------------------------------|-----|------|------|------------|-------|------|
| No visible manure contamination of feeding areas or water troughs            | 188 | 0.38 | 1.46 | 0.95, 2.26 | 0.084 | 4.6% |
| Trace amount of manure visible, feeding areas/water troughs cleaned > 1/week | 565 | REF  |      |            |       |      |
| Manure clearly visible, feeding areas/water troughs cleaned < 1/week         | 172 | 0.55 | 1.73 | 1.14, 2.63 | 0.010 | 7.6% |

---

**Q36. Calving area used for lame or sick cows?**

|                                                                |     |      |      |            |        |       |
|----------------------------------------------------------------|-----|------|------|------------|--------|-------|
| Calving area is never used by non-calving cows                 | 516 | REF  |      |            |        |       |
| Calving area is used by non-calving cows once in 3 months      | 125 | 0.05 | 1.05 | 0.63, 1.75 | 0.841  | 0.5%  |
| Calving area is used by non-calving cows at least once monthly | 75  | 0.28 | 1.32 | 0.73, 2.41 | 0.357  | 1.7%  |
| Calving area is used by non-calving cows at least once weekly  | 209 | 0.81 | 2.25 | 1.48, 3.42 | <0.001 | 14.2% |

---

**Q40. How quickly are calves removed from their dam?**

|                                             |     |      |      |            |       |       |
|---------------------------------------------|-----|------|------|------------|-------|-------|
| >90% are removed within 15 minutes of birth | 97  | REF  |      |            |       |       |
| >50% are removed within 30 minutes          | 507 | 0.84 | 2.32 | 1.23, 4.37 | 0.010 | 24.7% |
| 10-50% are removed within 30 minutes        | 236 | 0.42 | 1.52 | 0.76, 3.03 | 0.237 | 5.6%  |

|                                       |     |      |      |            |       |      |
|---------------------------------------|-----|------|------|------------|-------|------|
| <10% are removed within<br>30 minutes | 85  | 0.49 | 1.63 | 0.72, 3.7  | 0.239 | 3.2% |
| <b>Herd Growth</b>                    |     |      |      |            |       |      |
| Stable (<5%)                          | 256 | 0.23 | 1.26 | 0.78, 2.03 | 0.350 | 4.2% |
| Small Growth (5-25%)                  | 219 | 0.47 | 1.6  | 1, 2.56    | 0.049 | 6.7% |
| Medium (26 - 46%)                     | 218 | 0.41 | 1.51 | 0.95, 2.39 | 0.079 | 6.1% |
| Large (>46%)                          | 232 | REF  |      |            |       |      |

577

578 <sup>1</sup>n = number in category

579 <sup>2</sup>PAF = Population Attributable Fraction

580 <sup>3</sup>RA = Risk Assessment



581 **Table 5.** Results from final multivariable Bayesian beta regression model assessing the  
 582 association between RA questions, animal movement data and the probability of infection as  
 583 estimated by a Bayesian latent class analysis.

| Variable                                                                                                                            | n <sup>1</sup> | Coefficient | Probability of infection | 95% Probability Interval | Proportional reduction in R-squared |
|-------------------------------------------------------------------------------------------------------------------------------------|----------------|-------------|--------------------------|--------------------------|-------------------------------------|
| <b>Intercept</b>                                                                                                                    |                |             | 0.60                     | 0.25, 0.86               |                                     |
| <b>Q3. Presence of clinical JD or test positive cows in past</b>                                                                    |                |             |                          |                          |                                     |
| No and after testing                                                                                                                | 257            | REF         |                          |                          |                                     |
| No and before testing                                                                                                               | 348            | 0.52        | 0.72                     | 0.66, 0.77               |                                     |
| Yes and after testing                                                                                                               | 175            | 0.08        | 0.62                     | 0.57, 0.67               |                                     |
| Yes and before testing                                                                                                              | 145            | 0.44        | 0.70                     | 0.65, 0.75               |                                     |
|                                                                                                                                     |                |             |                          |                          | 22.6%                               |
| <b>Test Month</b>                                                                                                                   |                |             |                          |                          |                                     |
| January                                                                                                                             | 48             | 0.80        | 0.77                     | 0.69, 0.83               |                                     |
| February                                                                                                                            | 53             | 0.66        | 0.74                     | 0.66, 0.81               |                                     |
| March                                                                                                                               | 69             | 0.31        | 0.67                     | 0.59, 0.75               |                                     |
| April                                                                                                                               | 114            | 0.12        | 0.63                     | 0.55, 0.69               |                                     |
| May                                                                                                                                 | 160            | REF         |                          |                          |                                     |
| June                                                                                                                                | 129            | 0.10        | 0.62                     | 0.55, 0.69               |                                     |
| July                                                                                                                                | 91             | 0.49        | 0.71                     | 0.64, 0.77               |                                     |
| August                                                                                                                              | 67             | 0.37        | 0.68                     | 0.60, 0.76               |                                     |
| September                                                                                                                           | 53             | 0.38        | 0.69                     | 0.60, 0.76               |                                     |
| October                                                                                                                             | 69             | 0.63        | 0.74                     | 0.66, 0.80               |                                     |
| November                                                                                                                            | 44             | 0.87        | 0.78                     | 0.70, 0.85               |                                     |
| December                                                                                                                            | 28             | 0.35        | 0.68                     | 0.56, 0.78               |                                     |
|                                                                                                                                     |                |             |                          |                          | 35.3%                               |
| <b>Proportion of herd tested</b>                                                                                                    |                |             |                          |                          |                                     |
| Increase of 10%                                                                                                                     |                | -0.22       | 0.55                     |                          |                                     |
|                                                                                                                                     |                |             |                          |                          | 3.2%                                |
| <b>Q28. Are maiden or incalf heifers (≥6 months) fed forages that have received slurry from adult animals within the last year?</b> |                |             |                          |                          |                                     |
| No forages fed to heifers have been spread with slurry in the previous season                                                       | 50             |             |                          |                          |                                     |
| Fresh or conserved forages that were spread with slurry in the                                                                      | 875            | 0.35        | 0.68                     | 0.6, 0.76                |                                     |

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previous season are fed to  
heifers

---

1.3%

---

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**Q31. Dry cow cleanliness**

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No manure visible on hind legs or udder      135    0.28      0.67      0.61, 0.72

---

Manure present on hind legs but not above dewclaws      486    0.00      0.60

---

Manure present on hind legs but not above hocks, or is present on the udder or teats      213    0.14      0.63      0.59, 0.68

---

Manure present above the hocks      91    0.26      0.66      0.60, 0.72

---

7.1%

---

---

**Q36. Is the Calving Area ever used for lame or sick cows?**

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Calving area is never used by non-calving cows      REF

---

Calving area is used by non-calving cows once in 3 months      0.13      0.63      0.57, 0.69

---

Calving area is used by non-calving cows at least once monthly      0.14      0.63      0.56, 0.7

---

Calving area is used by non-calving cows at least once weekly      0.41      0.69      0.65, 0.74

---

5.8%

---

584

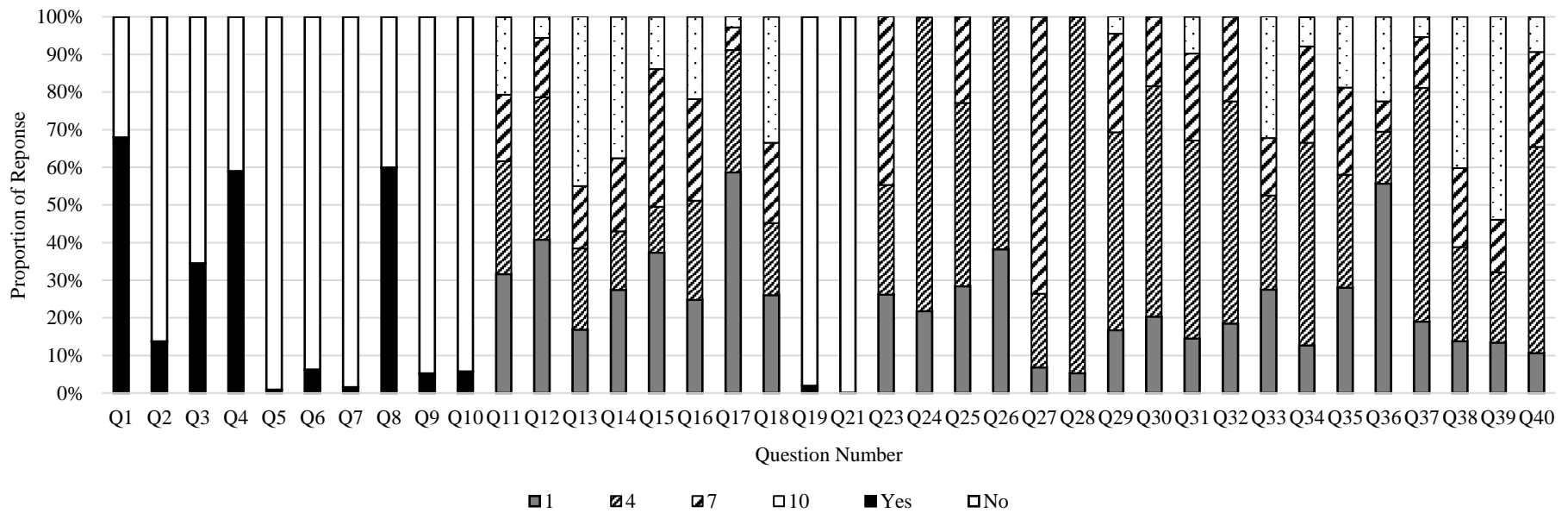
585    <sup>1</sup>n = number in each category

586

587 **McAloon Figure 1.** Stacked bar graph showing distribution of responses to Risk Assessment for 925 dairy herds enrolled in the Irish national  
588 Johne's Disease Control Programme. Questions 23-25 and Question 17 are scored to a maximum 7, questions 26 and 28 are scored to a  
589 maximum of 4.

590

591

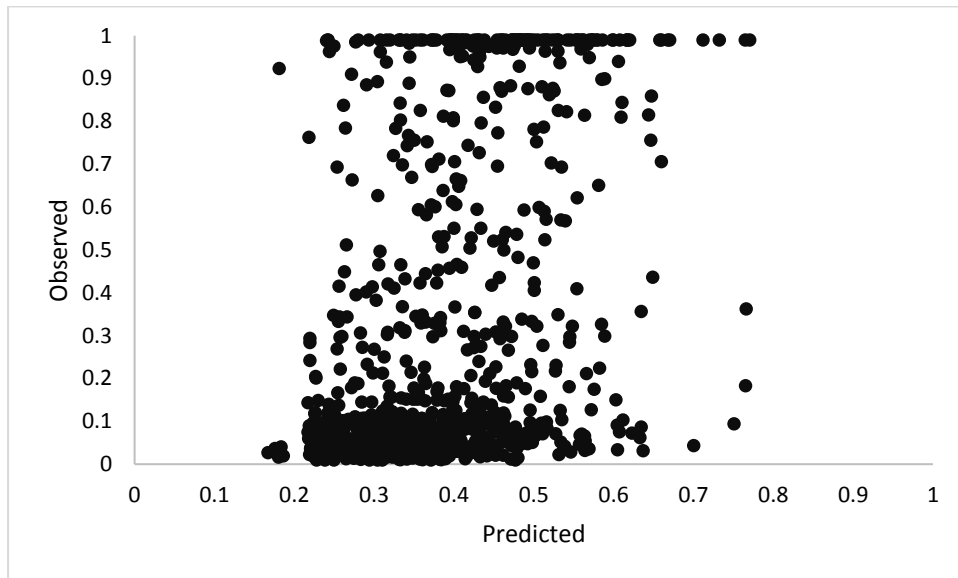


592

593 **McAloon Figure 2.** Predicted median probabilities of infection from final Bayesian beta  
594 regression model versus data outputs (observed) from Bayesian latent class model. R-squared  
595 = 0.16.

596

597



598